



clinical  
microbiology  
proficiency  
testing

## Mycology Plus

May 2006

### 0605-3 *Rhizopus* species

**HISTORY** Sent as a bronchial aspirate culture isolate.

**CMPT QA:** Pure growth of 4+ *Rhizopus* species, viable for 33 days.

**Reference Laboratory:** Growth of *Rhizopus* species confirmed.

See Table 1 for results. Nine participants correctly reported *Rhizopus* species and 1 reported *Absidia*. One laboratory reported the correct class, *Zygomycetes*, but speciation to genus level was expected. The taxonomic classification of *Rhizopus* is as follows: Kingdom, Fungi (*Myceteeae*); Phylum, *Zygomycota*; Class, *Zygomycetes*; Order, *Mucorales*; Family, *Mucoraceae*; Genus, *Rhizopus*.

Identification	No. of labs
<i>Rhizopus</i> species (1, refer)	9
<i>Zygomycetes</i> , refer	1
<i>Absidia</i> species	1
<b>Grand Total</b>	<b>11</b>

**ISOLATION** *Rhizopus* species grow on most media, but are inhibited by cyclohexamide<sup>1</sup>. Participants reported using a combination of media, e.g., SAB (n=5); BHI (6); IMA (5); PDA (3); Mycosel (1); Littman oxgall (1), Sabhi (1), and BAP (2). Incubation temperatures reported included 25°C, 28°C, 30°C, 37°C, and 42°C.

**IDENTIFICATION**<sup>1</sup> *Rhizopus* species are so named because they have rhizoids, which are root like anchors connected by hyphae called stolons that extend into the medium on which they are growing. Similar other organisms in the class *Zygomycetes* include *Absidia*, *Mucor*, *Apophysomyces*, *Rhizomucor*, *Saksenaea*, and *Cunninghamella*. The following can differentiate these genera: the length and location of their rhizoids, the diameter of sporangia, the shape of columellae, and the size, shape and surface texture of sporangiospores and the maximum growth temperature. Of these, only *Mucor* is without rhizoids. These same characteristics may be further used to differentiate between the *Rhizopus* spp. (e.g., *Rhizopus oryzae* (*arrhizus*), *Rhizopus microsporus* var. *rhizopodiformis*, and *Rhizopus stolonifer*<sup>1,2</sup>.)

**Colony morphology**<sup>1,2,3</sup> Colonies of *Rhizopus* are very fast growing, maturing within 4 days. The pathogenic species grow well at 37°C. *Rhizopus* quickly fills a Petri dish (agar surface) with a typically cotton candy like colony, initially white that turns grey to yellowish brown in time. The reverse is white to pale.

**Microscopic morphology** Like all members of the class *Zygomycetes* the hyphae are broad, those of *Rhizopus* measuring 6-15 µm in diameter, with few or no septa. Many stolons run among the mycelia, connecting groups of long (up to 4 mm) usually unbranched sporangiophores. The sporangiophores terminate with a dark, round sporangium (40-350 µm in diameter) that contains a columella and several oval, colourless or brown spores (4-11 µm in diameter). No collarette remains when the sporangial wall dissolves. In *Rhizopus* species, at the point where the stolons and sporangiophores meet the rhizoids are produced (directly opposite); in contrast, in *Absidia* the sporangiophores arise at points on the stolon that are between the rhizoids, not opposite them.

If non-septate hyphae (filaments) are seen in direct microscopic examination of bronchial lavage, bronchial washings or sputum, then *Rhizopus*, *Mucor* or *Absidia* must be considered.

In infected tissue, *Rhizopus* is visible as broad, thin-walled, hyaline, often aseptate or sparsely septate hyphae that are typically non-parallel and branched irregularly. Invasion of blood vessels is noteworthy<sup>4</sup>, but it is very difficult to isolate *Rhizopus* (and those other agents of zygomycosis/mucormycosis [see below]) from infected tissue and blood cultures. It is important to differentiate *Rhizopus* from *Aspergillus* as it also invades blood vessels<sup>2</sup>.

It is very important to note that regarding environmental airborne contaminants, a positive culture from a non-sterile specimen, such as sputum or skin, needs to be supported by direct microscopic evidence in order to be considered significant. Of course, a supporting clinical history in patients with appropriate predisposing conditions, is also helpful.

**CLINICAL SIGNIFICANCE** *Rhizopus* is a cosmopolitan filamentous fungus found in soil, house dust, old bread, decaying fruit and vegetables, and animal feces<sup>1</sup>. *R. oryzae* is distributed worldwide, but has a high prevalence in tropical and subtropical regions. It is used commercially to transform soybeans into edible products<sup>5</sup> and in the production of alcoholic beverages in Indonesia, China and Japan<sup>6</sup>.

While *Rhizopus* spp. are common contaminants, they are also occasional causes of serious (and often fatal) infections in humans. The fungus most commonly enters the body through the respiratory tract. Immunologically healthy people are capable of suppressing the growth of *Rhizopus* spp. and efficiently clear them from the lung<sup>4</sup>. Patients with diabetes mellitus, neutropenia, or those receiving corticosteroids are most at risk. AIDS does not appear to be a significant risk factor<sup>1</sup>. *Rhizopus* spp. are among the fungi causing the group of infections referred to as mucormycosis / zygomycosis. Although the term mucormycosis has often been used for this syndrome,

zygomycosis is now the preferred term for angio-invasive disease.

*Rhizopus oryzae* (*R. arrhizus*) is the most common causative agent of zygomycosis, accounting for some 60% of the reported culture positive cases, and nearly 90% of the rhinocerebral forms of infection<sup>6</sup>. *R. microsporus* var. *rhizopodiformis* is the second most frequently isolated zygomycete, accounting for between 10-15% of reported human cases, especially from cutaneous and gastrointestinal infections.

**TREATMENT**<sup>3</sup> Similar to the other genera belonging to the phylum *Zygomycota*, treatment of *Rhizopus* infections remains difficult. Due to its property to invade vascular tissues, infarction of the infected tissue is common and mortality rates are very high. Early diagnosis is crucial and surgical debridement or surgical resection, as well as antifungal therapy, is usually required. Amphotericin B is the most commonly used antifungal agent. Clinical response to therapy is frequently unsatisfactory in zygomycosis. Reversal of immunosuppression is one of the most significant factors influencing the clinical outcome. Interestingly, fluconazole in combination with trovafloxacin or ciprofloxacin proved to be effective in a murine model of pulmonary zygomycosis.

## REFERENCES

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6. <http://www.mycology.adelaide.edu.au/Mycoses/Oppportunistic/Hyalohyphomycosis/index.html>